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(54) Title: PHOSPHATE DERIVATIVE SUPPLEMENTS

(57) Abstract: There is provided a supplement comprising one or more phosphate derivatives of electron transfer agents and having an enteric coating.

Phosphate Derivative Supplements

Field of the invention

The invention relates to supplements containing phosphate derivatives of electron transfer agents. More particularly, the invention relates to supplements containing phosphate derivatives of electron transfer agents and having an enteric coating.

Background of the invention

In this specification, where a document, act or item of knowledge is referred to or discussed, this reference or discussion is not an admission that the document, act or item of knowledge or any combination thereof was at the priority date:

(i) part of common general knowledge; or

(ii) known to be relevant to an attempt to solve any problem with which this specification is concerned.

Whilst the following discussion concerns tocopherol and ubiquinol, it is to be understood that this is merely illustrative and that the invention is not limited to tocopherol and ubiquinol but that the invention also similarly relates to the other electron transfer agents.

The absorption of poorly water soluble, lipophilic substances following oral administration is generally low and highly variable due to their low solubility within the gastrointestinal tract and the poor dissolution and dispersion properties of conventional formulations for compounds of this nature. This can impact on the pharmaceutical utility of such compounds. Variation in bioavailability demonstrated by lipophilic substances may limit their use in medicine as the required dosage is unpredictable. If a dose could be predictably provided to humans and animals, the utility of these lipophilic substances would be greatly increased.

In order for pharmaceutical drugs and nutrients to be valuable in clinical practice, they must exhibit certain properties. For example, they must display adequate stability, solubility and permeability. Free tocopherol is unstable and therefore unsatisfactory as a bioavailable form of Vitamin E.

Derivatisation has long been recognized as an important means of increasing efficacy and bioavailability. However, derivatised pharmaceutical drugs and nutrients must then further exhibit capability to revert to the parent compound once absorbed into the systemic circulation in order to be useful.

One example of a derivatised pharmaceutical drug or nutrient is the current use of vitamin E organic esters, typically acetate or succinate. In the alimentary canal, it has been found that there is lipase activity that releases free tocopherol from the esters of tocopherol, typically the acetate ester. This property permits a formulator using tocopheryl acetate as a nutritional source of Vitamin E, and avoids the instability of free tocopherol.

The salts of tocopheryl phosphate have been known to be water-soluble for some time. These properties have led to research on the use of alpha tocopheryl phosphate as a very bioavailable form of Vitamin E activity for supplementation. Tocopheryl phosphate has been shown to have superior bioavailability compared to ester derivatives, therefore the presence of the phosphate group on the tocopherol molecule may be important to bioavailability.

However, even when tocopheryl phosphate is used, there is a need for improved absorption to achieve efficiency and reproducible results.

Coenzyme Q_{10} (CoQ_{10}) (also known as ubiquinone) is an essential lipophilic compound required for many bodily functions by both humans and animals. In particular, it is a component of the mitochondrial respiratory chain, where it acts as an electron carrier. CoQ_{10} also functions as a lipid soluble antioxidant in its reduced form (ubiquinol). It has been suggested that supplementary CoQ_{10} may also protect against LDL oxidation and reduce free radical damage in certain patients. CoQ_{10} is a highly lipophilic (calculated log P ~ 20), poorly water soluble (aqueous solubility < 0.1 μg/ml) compound that is subject to poor and variable absorption properties following oral administration. As stated above, this significantly limits its clinical utility.

Attempts to prepare derivatives of CoQ_{10} in conditions appropriate for commercial production have not been successful. There is still a need for a formulation which will deliver CoQ10 with improved bioavailability.

Therefore, there is still a need for a form of tocopheryl phosphate, CoQ_{10} and other electron transfer agents which can be taken orally.

Summary of the Invention

It has been surprisingly found that the poor bioavailability of tocopheryl phosphate and other phosphate derivatives of electron transfer agents is not because the phosphate derivatives are cleaved by the phosphatases in the stomach exposing the electron transfer agent to be degraded. The amount of phosphate derivatives which are cleaved is less than 5%.

It has further been surprisingly found that the use of an enteric coating is nevertheless an effective delivery system improving bioavailability of phosphate derivatives of electron transfer agents.

Whilst not wishing to be bound by theory, the improved bioavailability is thought to be due to fact that the enteric coating minimizes the formation of the insoluble forms of the phosphate derivatives. The phosphate derivatives of electron agents must be solubilised in order to be absorbed by the body. The enteric coating improves delivery of the phosphate derivatives of electron transfer agents to the small intestine and thus improves bioavailability of the compound.

The term "electron transfer agent" is used herein to refer to the class of chemicals which may be phosphorylated and which (in the non-phosphorylated form) can accept an electron to generate a relatively stable molecular radical or accept two electrons to allow the compound to participate in a reversible redox system. Examples of classes of electron transfer agent compounds that may be phosphorylated include hydroxy chromans including alpha, beta and gamma tocols and tocotrienols in enantiomeric and raecemic forms; quinols being the reduced forms of vitamin K1 and ubiquinone; hydroxy carotenoids including retinol; and ascorbic acid.

The term "phosphate derivatives" is used herein to refer to the acid forms of phosphorylated compounds, salts of the phosphates including metal salts such as sodium, magnesium, potassium and calcium and any other derivative where the phosphate proton is replaced by other substituents such as ethyl or methyl groups or phosphatidyl groups.

The word 'supplement' as used in this description refers to all forms of supplying pharmaceuticals or nutrients orally or enterally to humans or animals. For example, tablets, powders, chewable tablets, capsules, oral suspensions, children's formulations, enteral feeds, nutraceuticals, and functional foods.

According to the invention, there is provided a supplement comprising one or more phosphate derivatives of electron transfer agents and having an enteric coating.

Examples of phosphate derivatives of electron transfer agents include ubiquinyl phosphate, retinyl phosphate, ascorbyl phosphate, menadiol disodium phosphate, tocopheryl phosphate, di-tocopheryl phosphate, tocopheryl pyrophosphate, di-tocopheryl pyrophosphate in their acid form or as acceptable salts or mixtures thereof.

Preferably, where the supplement comprises phosphate derivatives of tocols, the supplement also contains free tocopherol. The tocol phosphate derivatives act as an emulsifier and assist the absorption of free tocopherol.

The enteric coating must be insoluble in the stomach (low pH) and survive the enzymes in saliva, but degrade in the absorption site which is just after the stomach at a pH greater than 6. Typically, the coating is a water soluble polymer. For example, it may be a cellulose ether, polyvinylpyrrolidone or polyethylene glycol.

The release of the phosphate derivatives of electron transfer agents is typically delayed for at least 10 to 30 minutes to ensure maximum re-dissolution of the phosphorylated derivative of the electron transfer agent. Generally, delays of more than 1 hour are not desirable as it is necessary for the supplement to be released in the proximal small intestine to maximise its usefulness. Enteric coatings which cause a delay of more than 1 hour may result in release after the distal small intestine and are not likely to be suitable for use with this invention. However, a person skilled in the art will realize that each person's digestive system functions differently and these timings are merely illustrative. The important feature is that the enteric coating must be insoluble in the stomach (low pH) and survive the enzymes in saliva, but degrade in the absorption site which is just after the stomach at a pH greater than 6.

In one preferred embodiment, the supplement will be in the form of a tablet, capsule, cross-linked soft or hard gelatin capsule. The tablet or capsule will preferably be film coated with cellulose or methylcellulose or a similar substance designed to delay release of the active ingredients. Enteric coating agents are used to protect the tablet core and phosphate derivatives of electron transfer agents from reacting with characteristic dissolution factors of the different regions of the gastrointestinal tract. Polymers with pH dependant solubility properties (enteric coatings) have been found to be most useful for this application. Cross-linking of gelatin in the hard or soft gelatin capsule is understood to impart the same biological effect.

In a second preferred embodiment, the phosphate derivatives of electron transfer agents may be spray dried to form micro-particles and then the micro-particles enterically coated. These enteric-coated micro-particles can then be used in a range of pharmaceutical and food dose forms including hard gelatin capsules, oral suspensions, children's formulations, enteral feeds and functional foods.

Phosphate derivatives of electron transfer agents and their metabolites are absorbed through the lining of the intestine into the portal vein and/or lymph eventually reaching the arteries, tissue and organs. Once in circulation the phosphate derivative of electron transfer agents and their metabolites can provide therapeutic benefits to the human recipient. It is anticipated that the use of an enteric coating will increase the amount of the phosphate derivatives electron transfer agents available for absorption in the small intestine and increase beneficial effects.

According to a further aspect of the invention, there is provided a method for improving the bioavailability of one or more electron transfer agents comprising the step of applying an enteric coating to a supplement comprising one or more phosphate derivatives of one or more electron transfer agents.

According to another aspect of the invention, there is provided a method for minimizing the formation of insoluble forms of phosphate derivatives of electron transfer agents comprising

(a) preparing a supplement comprising one or more phosphate derivatives of one or more electron transfer agents; and

(b) applying an enteric coating to the supplement.

According to another aspect of the invention, there is provided a method for improving bioavailability of one or more electron transfer agents comprising the step of delivering a supplement to the small intestine in a form such that one or more phosphate derivatives of one or more electron agents are released in the small intestine.

According to another aspect of the invention, there is provided a method of making a dosage form of a phosphate derivative of one or more electron transfer agents comprising:

(a) preparing a supplement comprising one or more phosphate derivatives of one or more electron transfer agents; and

(b) applying an enteric coating to the supplement.

According to another aspect of the invention, there is provided a method for minimising the formation of insoluble forms of phosphate derivatives of electron transfer agents comprising:

(a) preparing a supplement comprising one or more phosphate derivatives of one or more electron transfer agents; and

(b) applying an enteric coating to the supplement;

such that upon oral or enteral application of the supplement to a patient the phosphate derivatives of one or more electron transfer agents are made available for absorption thought the walls of the small intestine.

Detailed Description of the Invention

Those skilled in the art will recognize that several dose forms and enteric strategies are available to delay release of bioactive compounds, including cross-linked hard gelatin capsules, micro-encapsulation, enteric coated tablets, and enteric coated soft gelatin capsules. These delivery strategies should minimize contact with gastrointestinal phosphatases and acids to prevent cleavage of the phosphate group and formation of insoluble dihydrogen phosphates. Controlled release of the phosphate derivatives of electron transfer agents can therefore improve absorption and bioactivity by increasing the volume of active substance released into the small intestine being one of the primary sites for nutrient and drug absorption.

This technology could not be used for ester derivatives or bioactive molecules that rely on acidic degradation in the upper stomach to remove an ester or other group releasing the free compound for absorption further down the gastrointestinal tract.

The purpose of the enteric coating strategy is to confer greater bioavailability of the electron transfer agents. It would therefore be expected that release of phosphate derivatives of electron transfer agents by an enteric dose form strategy at the site of absorption in the small intestine, alone explains the observed increase in blood levels seen with oral administration of enteric coated forms compared to regular dose forms.

The phosphate derivatives of electron transfer agents are put into a tablet or capsule form using any methods known to those skilled in the art. It will be readily understood by those skilled in the art that phosphate derivatives of electron transfer agents can be put in tablet or capsule form in a number of different ways. It will be understood that a variety of different binders, fillers and a number of other excipients can be used.

An enteric coating is then applied to the tablet or capsule by usual methods. The enteric coating may include cellulose, methylcellulose or a derivative of either of these or another similar substance designed to delay the release of the active ingredients until they reach the region of the small intestine having a pH greater than 6. It is also possible to place the phosphate derivatives of electron transfer agents in a capsule which has been enterically coated or cross-linked. Each of these methods will delay the release of the phosphate derivatives of electron transfer agents until they reach the region of the small intestine having

a pH greater than 6. Typically the maximum thickness of the enteric coating will be 0.5 mm or otherwise adequate to allow the dose form to comply with established USP 2000 standards for delayed release dose forms.

When the tablet, capsule or micro-particle as detailed above, is swallowed, the dose form moves down the digestive tract to the stomach. As the enteric coated tablet or capsule moves down the upper alimentary tract, the outer surface coating remains intact as a coating but changes chemical form creating insoluble acid complexes. Although some of the enteric coating is dissolved or abraided in the upper alimentary tract, the majority of the coating adheres to the tablet, micro-particle or capsule core until reaching the more alkaline small intestine. At this point the enteric coating completely dissolves releasing content of the dose form.

Accordingly, the enteric coating ensures that the phosphate derivatives of electron transfer agents are not released until the small intestine, where it will be most effective. Phosphate derivatives of electron transfer agents and/or their metabolites are absorbed through the lining of the intestine into the lymphatic and/or portal vein eventually reaching the arteries, tissue and organs. Once in circulation phosphate derivatives of electron transfer agents and/or their metabolites can provide therapeutic benefits to the human recipient.

Examples

The invention will now be further illustrated and explained in the following non-limiting examples.

Comparative Example 1

In this example, tocopheryl phosphate which was exposed to various environments to ascertain whether the phosphate group would be readily cleaved.

(a) HCl at 1M

10g of di-hydrogen tocopheryl phosphate was dispersed in 50 ml of water and the pH adjusted to 1.0 with hydrochloric acid. The mixture was heated at 60 to 70°C for two hours then analyzed for tocopherol (by electrospray mass spectrometry) and phosphate, and less than 1% was detected, that is, less than 1% of the tocopheryl phosphate was cleaved.

(b) Phosphatase in an acidic medium

50 mg of di-sodium tocopheryl phosphate was dissolved in 100 ml 0.1 M phosphate buffer with pH 4.8. 25 units of acid phosphatase was added and then the temperature was held at

37°C for 22 hours. Less than 3% of free tocopherol was detected by electrospray mass spectrometry, that is, less than 3% of the tocopheryl phosphate was cleaved.

(c) Phosphatase in an alkaline medium

1g of di-sodium tocopheryl phosphate was dissolved in 150 ml 1M diethanolamine buffer pH 9.8. 87 mg of alkaline phosphatase was added and then the temperature was held at 37°C for 18 hours. Less than 1% of free tocopherol was detected by electrospray mass spectrometry, that is, less than 1% of tocopheryl phosphate was cleaved.

These results show that tocopheryl phosphate is not cleaved in environments which are acidic, even in the presence of phosphatases. Therefore, the low bioavailability of current tocopheryl phosphate treatments is not due to cleavage of tocopheryl phosphate in the stomach.

Comparative Example 2

In this example ubiquinyl phosphate was exposed to acidic conditions similar to that of the stomach to ascertain whether the phosphate group would be readily cleaved.

Ubiquinyl phosphate was dispersed in a mixture of propionic acid and water having a pH of 1.0 at a temperature of 37°C. The mixture was then analyzed by ^{31}P NMR for free phosphate and ubiquinyl phosphate. The amount of free phosphate was negligible. The mixture was analyzed again 3 hours later and there was still only a negligible amount of free phosphate. Therefore, only a negligible amount of ubiquinyl phosphate was cleaved in acidic conditions similar to that of the stomach.

Example 1 Enteric coated tocopherol phosphate tablet

One form of tocopherol phosphate which can be used in the supplement is α-tocopherol phosphate powder although other forms may be possible. Although it is possible to use other tocopherol isomers in the supplement, it will be understood that the α-tocopherol isomer has been shown to have significantly higher bioavailability than other tocopherol sources which may not provide the same health benefits due to inadequate plasma or tissue levels.

<u>Tablet core</u>

It will be readily understood by those skilled in the art that tocol phosphate derivative powder can be formulated a number of different ways. It will be understood that a variety of different binders, fillers and a number of other excipients can be used providing that the tablet core performs to the relative dissolution and disintegration standards published, for example, in the

United States Pharmacopeia (USP 2000). Depending upon the dose required an uncoated tablet core may be formulated as following:

Component	Grade	%w/w
Disodium tocopheryl phosphate	Spray dried	45
Microcrystalline cellulose	Avicel M101	10
Dicalcium phosphate	Di Tab	25
Magnesium stearate	HY Qual BP	15
Crospovidone	NF XL-10	5

These are made in small batches for experimental trials (1-2kg) or larger batches for commercial production (500-600,000 tablets).

Enteric coating

It will be recognized by those skilled in application of enteric coatings that several polymer and coating products can be used to form an effective enteric film coating. For example, protective polymers with free carboxyl groups are insoluble in acid solutions and dissolve by salt formation at a pH greater than 6. Examples of such coatings include but are not limited to cellulose acetate pthalate, hydroxypropyl methylcellulose pthalate, carboxymethyl-ethylcellulose, sodium alginate and polyvinyl acetate phthalate (Opadry®, Pharmacoat®, Methocel®, Sepifilm®, Eudragit® respectively) presented as both aqueous and organic coating formulations. Numerous formulation approaches are available depending upon the film-forming polymer utilized and peculiarity of the tablet core.

Formulation 1

Component	%w/w
Opadry®	73
Talc or similar lubricant	20
Pigment (TiO_2)	7

Solids content 20% & coating quantity 1-5mg/cm^2

Formulation 2

Component	%w/w
Pharmacoat®, Methocel®	80
PEG 6000	8
Talc or similar lubricant	5
Pigment (TiO_2)	7

Solids content 12% & coating quantity 1-5mg/cm^2

Formulation 3

Component	%w/w
Sepifilm®	93
Pigment (TiO_2)	7

Solids content 18% & coating quantity 1-5mg/cm^2

Since powders of cellulose ethers tend to form lumpy solutions the Opadry®, Pharmacoat®, Methocel®, or Sepifilm® is first suspended in hot water then cooled with stirring. The pigment is mixed separately in suitable high-shear homogeniser and added just before use. If excessive foaming occurs during mixing, add an anti-foam emulsion (sorbitan sesqui oleate 0.0025%).

After de-dusting, the tablet cores are loaded into a suitable conventional coating pan or fluidized bed coater (Hi-Coater, Rapid Coater, Kugelocoater etc) and pre-heated to 35 to 40°C with limited agitation to prevent attrition. A first small portion of coating solution is then applied very slowly to impart mechanical stability to the tablet core surface and reduce dust formation which may impair quality and performance of the finished film. The heated air supply is then increased and the coating solution sprayed continuously onto the rapidly moving tablet core bed. Inlet air temperatures are initially between 60 to 90°C, but may vary depending upon the spray rate, water content of the inlet air source and efficiency of the particular equipment utilized. Each 10 µm of film coat usually takes approximately one hour

to apply using a pan coater and less than half this time in a fluidized bed coater. The weight gain of a 400 gm tablet is approximately 1% and the tensile strength of the finished film coat is typically 20 to 45 N/mm^2 but may vary from 10 to 55 N/mm^2 depending upon the cellulose derivative used. Accordingly, if alternative polymers are utilized this variation may be even wider. If a higher gloss appearance is required a final coat of pure polymer may be applied.

Example 2 Enteric coated tocopherol phosphate hard and soft gelatin capsules

Gelatin capsules can be coated to impart resistance to gastric fluid, improve stability to moisture and heat and utilized to deliver tocol phosphate derivatives. Cross linking of gelatin in the capsule has also been suggested as a means of delaying capsule degradation.

Although application of the film coating is basically similar to methods utilized for tablets, some additional problems are experienced due to the nature of capsule. Low porosity of the gelatin matrix reduces adhesion of the film to the capsule surface and can cause differences in elasticity of the coating polymer film and the capsule. This can cause the capsule to burst especially when placed under mechanical stress experienced for example, when the capsule is squeezed out of blister packaging. To reduce this "eggshell' effect, a coating emulsion with water based solvent is utilized. The water contained in the coating solution causes the gelatin to swell slightly, allowing the film to bond to the gelatin and adhere more effectively. This process prevents the capsule from embrittlement and with adaptation can be used for starch capsules.

Suitably sized hard gelatin capsules (eg; Snap fit, Capsugel, Basel) are filled with a mixture of disodium α-tocopherol phosphate and suitable flow agents such as magnesium stearate etc. The tocopherol powder mixture is then fed into the larger of the two capsule halves by hand or mechanically in a capsule filler and band sealed again by hand or mechanical means. The capsule can be coated with one of several film-forming polymers to delay absorption of the tocopherol until the alkaline small intestine.

The enteric coating may include the cellulose derivatives previously mentioned, and formulation examples follow:

Formulation 4

Component	%w/w
Eudragit® L 30 D-55 (*30% solids)	12.40
Triethyl citrate	1.20
Talc or lubricant	6.20
Water	80.20

Solids content 19.80% & coating quantity 16.70mg/cm^2

Formulation 5

Component	%w/w
Eudragit® L 30 D-55 (*30% solids)	9.30
Triethyl citrate	1.40
Talc or lubricant	5.00
Pigment (TiO_2)	4.30
Water	80.00

Solids content 20.00% & coating quantity 5.70mg/cm^2

The capsules are tipped into a suitable coating aparatus (Hi-Coater, Rapid Coater, Kugelocoater etc) and core temperature raised to and maintained at 30 to 32°C during application of the film coat. Following the same instructions for tablets, the enteric coating is then sprayed onto the capsule. After enteric coating application the capsules are then conditioned at 50% relative humidity and cooled. After manufacture, the gelatin capsules can withstand up to 65N in a hardness tester and starch capsules up to 208N. Shelf life at 37°C can extend to 2 years and disintegration in intestinal fluid ranges from 5 to 35 minutes.

Example 3 Enteric coated tocopherol phosphate micro-particle encapsulation and enteric delivery

Alginate micro-encapsulation and lecithin vesicular technology can also be used as enteric delivery strategies to delay particle release until the small intestine.

In alginate micro-encapsulation, the alginate is typically used in the sodium form and provides protection from gastric acid by converting to the insoluble alginic acid during transit in the stomach. After passage through this acidic environment the alginic acid converts back to the soluble sodium form as the pH is raised upon entry to the small intestine. Contents of the tablet or micro particle are therefore protected at acidic pH and only released at alkaline pH of the small intestine. While the alginate may be utilized as the primary film former, combinations with synthetic film formers such as polyvinyl acetate phthalate are also known.

Micro-encapsulation is used to entrap a wide variety of substances in the pharmaceutical, cosmetic and agricultural industries where delayed release is required in a micro-particle size. Calcium alginate entrapment is particularly preferred because the process is expeditious, the manufacturing conditions are mild and non toxic reactants are employed. Other micro-encapsulation materials can also be employed to control release of the active including gellan gum and gum arabic but not recommended due to the need for higher temperatures during manufacture.

Water soluble forms of tocol phosphate derivatives in the required dose are mixed in a 1-2% solution of sodium alginate using a suitably sterile, pure water source. Effective preservation is achieved by addition of sodium benzoate at a concentration of 1000 ppm and clean manufacturing facilities. The solution is then pumped through a small orifice (such as a needle) and allowed to free fall for sufficient time to form a spherical bead. Size of this bead may be varied according to the size of the needle orifice, volume discharged and solution viscosity. The spherical bead drops into a 1-2% calcium chloride solution or suitable di or tri-valent ion source, and gels upon contact remaining in the bath until hardened. Excess calcium ion solution is then rinsed from the bead and beads screened for size.

A number of variations are possible with this procedure including use of an emulsion and manipulation of various physico-chemical properties to alter shape, size and permeability of the microcapsule.

Release of the tocol phosphate derivative is generally rapid and corresponds to the high water solubility of the tocol phosphate derivative. Accordingly release kinetics can be altered by

using different tocol phosphate derivatives with higher dissolution possible with disodium salts and slower release with the monosodium salts.

Example 4 Ubiquinyl phosphate

In this example, ubiquinyl phosphate was prepared to be used in supplements having enteric coatings according to the invention.

100g ubiquinol was heated to 100 °C and 33g of P_4O_{10} was added. The mixture was stirred for 3 hours and 500 ml water was then introduced slowly into the mixture. The temperature of the reaction was maintained just below boiling point for a further 1 hour. Removal of water yielded ubiquinyl phosphate, and inorganic phosphates. The inorganic phosphates were removed by further washes with hot water. The remaining amorphous material was then mixed with 100 L of virgin grade canola oil containing at least 1 to 5% lecithin. The final mixture therefore provides 1mg/ml and can be dispensed to give the required dose. The amorphous ubiquinyl phosphate can also be spray dried using conventional methods to form a buff coloured powder which can be sieved to the required mesh size.

Example 5

Equivalent to Example 1 substituting ubiquinyl phosphate powder for tocopheryl phosphate.

Example 6

Equivalent to Example 2 using ubiquinyl phosphate in canola oil for tocopheryl phosphate.

Example 7

Equivalent to Example 3 substituting ubiquinyl phosphate powder for tocopheryl phosphate.

Example 8

The aim of the study was to dose rats with deuterated tocopheryl phosphate or deuterated tocopheryl acetate by oral administrations, then determine the amounts of tocopheryl phosphate in livers following treatment.

The procedure used is summarized as follows:

1. Administer a single dose of the deuterated compounds to male Sprague-Dawley rats (see table; oral gavage with an 18g gavage needle and 1 ml syringe, or intravenously with a 26g hypodermic needle and 1 ml syringe).

2. Twenty-four hours after administrations the rat will be anaesthetized with Nembutal (60 mg/kg i.p.).

3. Once the rats are under deep anaesthesia a sample of blood will be taken from the tail vein, and the femoral vein exposed and injected with 500 units of heparin. The abdominal cavity will be opened and the rat perfused with saline. The liver, heart and epidydimal fat pad removed and frozen in liquid nitrogen. Hind-leg muscle and brain will also be collected and frozen.

Table 1: Treatment options

Compound	Dose (mg/kg)	Number of Rats
Tocopheryl phosphate (IV)	10	3
Tocopheryl phosphate (IV)	30	3
Tocopheryl phosphate	30 (enteric coated)	3
Tocopheryl phosphate	10	3
Tocopheryl phosphate	30	3
Tocopheryl acetate	10	3
Tocopheryl acetate	30	3
Tocopheryl acetate	100	3
Control (0.3 ml water)(IV)	0	3
Control (0.3 ml corn oil)	0	3

The enteric coated doses were prepared as per Example 3 above in order to prepare tablets of an appropriate size for the rats.

Livers were be extracted according to the method below. The extracts will be analyzed and quantitated for tocopheryl phosphate (μg) content by ES MS. Any tissue samples left over at the end of the study was kept frozen at –80 °C.

Liver extraction.

1 g liver was homogenized in 10 ml dichloromethane (analytical grade). Add 0.1 mg tocopheryl diphosphate (internal standard). Homogenize sample for 2 min, centrifuge and

remove upper layer and evaporate under nitrogen. Add 9 ml KOH (2M) and stir for 1 hr at room temperature (or 20 min at 80 C). Add 10 ml hexane, shake and remove upper layer. Add 10 ml HCl (2M) and shake. Add 10 ml hexane to the solution and shake and remove upper layer. Evaporate top layer to dryness.

Electrospray analysis:

Add 1 ml tetrahydrofuran (THF) and 20 ul of 25% Ammonia to sample and analyse.

Results

Treatment	Control	10 mg/kg	30 mg/kg	100 mg/kg
Tocopheryl phosphate (IV)	6.6 ug	14.4 ug (94%)	15.4 ug (100%)	15.0 ug
tocopheryl phosphate enteric coated	6.6 ug		12.5 ug (81%)	
tocopheryl phosphate oral	6.6 ug	8.0 ug (52%)	10.0 ug (65%)	
tocopheryl acetate oral	6.6 ug	10.6 ug (69%)	10.0 ug (65%)	12.0 ug

Tocopheryl phosphate was administered by IV to provide a value for absolute bioavailability. The amounts in brackets are percentages when compared with the IV value.

Conclusion

The above results are quite surprising and clearly demonstrate that:

1. absorption of a tocopherol analogue can improve tissue levels within a 24 hour period;
2. enterically coated tocopheryl phosphate has higher bioavailability than both uncoated tocopheryl phosphate and tocopheryl acetate. The 30 mg/kg enteric coated tocopheryl phosphate supplement achieved similar results to the 100 mg/kg tocopheryl acetate dose indicating that the tocopheryl phosphate enteric coated dose is approximately three times more bioavailable than tocopheryl acetate to liver tissue.

The word 'comprising' and forms of the word 'comprising' as used in this description and in the claims does not limit the invention claimed to exclude any variants or additions.

Modifications and improvements to the invention will be readily apparent to those skilled in the art. Such modifications and improvements are intended to be within the scope of this invention.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A supplement comprising one or more phosphate derivatives of one or more electron transfer agents and having an enteric coating.

2. A supplement according to claim 1 wherein the one or more phosphate derivatives of one or more electron transfer agents are selected from the group consisting of ubiquinyl phosphate, retinyl phosphate, ascorbyl phosphate, menadiol disodium phosphate, tocopheryl phosphate, di-tocopheryl phosphate, tocopheryl pyrophosphate, di-tocopheryl pyrophosphate in their acid form or as acceptable salts or mixtures thereof.

3. A supplement according to claim 2 wherein the electron transfer agents are selected from the group consisting of tocopheryl phosphate, di-tocopheryl phosphate, tocopheryl pyrophosphate, di-tocopheryl pyrophosphate in their acid form or as acceptable salts or mixtures thereof.

4. A supplement according to claim 3 wherein the supplement further comprises free tocopherol.

5. A supplement according to claim 1 wherein the enteric coating delays release of the phosphate derivatives of the electron transfer agents until they reach the region of the small intestine having a pH greater than 6.

6. A supplement according to claim 1 wherein the supplement is in the form of a tablet, capsule, cross-linked soft or hard gelatine capsule.

7. A supplement according to claim 1 wherein the phosphate derivatives of the electron transfer agents are spray-dried to form micro-particles and then the micro-particles enterically coated.

8. A supplement comprising one or more phosphate derivatives of one or more electron transfer agents selected from the group consisting of tocopheryl phosphate, di-tocopheryl phosphate, tocopheryl pyrophosphate, di-tocopheryl pyrophosphate in their acid form or as acceptable salts or mixtures thereof and having an enteric coating.

9. A supplement comprising one or more phosphate derivatives of ubiquinol and having an enteric coating.

10. A supplement in the form of a tablet, capsule, cross-linked soft or hard gelatine capsule comprising one or more phosphate derivatives of one or more electron transfer agents and having an enteric coating.

11. A method for minimizing the formation of insoluble forms of phosphate derivatives of electron transfer agents comprising:

(a) preparing a supplement comprising one or more phosphate derivatives of one or more electron transfer agents; and

(b) applying an enteric coating to the supplement.

12. A method for improving bioavailability of one or more electron transfer agents comprising the step of delivering a supplement to the small intestine in a form such that one or more phosphate derivatives of one or more electron agents are released in the small intestine.

13. A method of making a dosage form of a phosphate derivative of one or more electron transfer agents comprising:

(c) preparing a supplement comprising one or more phosphate derivatives of one or more electron transfer agents; and

(d) applying an enteric coating to the supplement.

14. A method for minimising the formation of insoluble forms of phosphate derivatives of electron transfer agents comprising:

(c) preparing a supplement comprising one or more phosphate derivatives of one or more electron transfer agents; and

(d) applying an enteric coating to the supplement;

such that upon oral or enteral application of the supplement to a patient the phosphate derivatives of one or more electron transfer agents are made available for absorption thought the walls of the small intestine.

15. A method for improving the bioavailability of one or more electron transfer agents comprising the step of applying an enteric coating to a supplement comprising one or more phosphate derivatives of one or more electron transfer agents.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01206

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 31/665, 9/28, 9/40, 9/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC AS ABOVE

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU: IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPAT, CAPLUS; tocol,phosphate, tocopheryl()phosphate, enteric, tocopheryl(w)pyrophosphate, ubiquinol, retinyl()phosphate, ascorbyl()phosphate, menadiol()phosphate.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 699440A (Senju Pharmaceutical Co., Ltd) 6 March 1996. See Formulation Example 1 page 6.	1-8, 10-15
X	EP 679399A (Senju Pharmaceutical Co., Ltd) 2 November 1995. See Formulation Example 4 page 5.	1-8, 10-15
X	EP 661053 (Senju Pharmaceutical Co., Ltd) 5 July 1995. See Formulation Example 4 page 8.	1-8, 10-15

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 November 2001	15 NOV 2001
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer G.R.PETERS Telephone No : (02) 6283 2184

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01206

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 324387A (Takeda Chemical Industries LTD and Senju Pharmaceutical Co., Ltd) 19 July 1989. See example 3 page 5.	1-8, 10-15
X	US 5114957 (Hendler S. et al) 19 May 1992. See example II column 5.	1-8, 10-15

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/01206

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
EP	699440	CA	2152693	JP	8099883	US	5650404
EP	679399	JP	8003049				
EP	661053	CA	2139209	JP	7196516	US	5489576
EP	324387	CA	1328810	PH	25859	US	

END OF ANNEX